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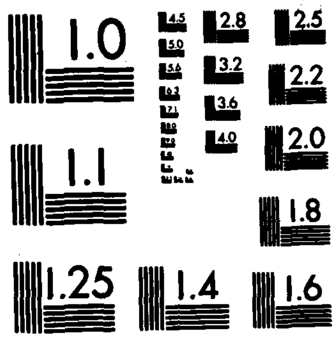

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NEUROPHYSIOLOGICAL STUDY OF VECTOR RESPONSES TO REPELLENTS

Annual Report

Francis T. Takahashi, Jr.
and
Edward E. Davis

August 1979

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Nectar- and blood-feeding and oviposition behaviors, which are important to the survival of the mosquito, depend heavily on the detection of airborne chemical signals. The objective of this research is to gain a better understanding of the basic mechanisms of chemoreception and the action of repellents on the sensory system, thus facilitating the development of more efficient means for interfering with the detection of the host or otherwise altering the behavior of the vector.

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19. KEY WORDS (Continued)

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The overall aim is to protect military personnel against vector-borne diseases.

The comparison of the responses of the five different types of chemosensory sensilla on the antennae of _Aedes aegypti_ indicated that these different morphologic types each responded to a series of host odors, oviposition attractants, and repellents with characteristic patterns of electrophysiologic responses. Each receptor type responded to at least a few repellents, hence weakening the case for a specific nociceptor sensilla which would be sensitive to noxious stimuli and repellents. Interspecific comparisons of electrophysiologic responses of _A. triseriatus_, _Culex tarsalis_, and _Anopheles stephensi_ with those of _A. aegypti_ revealed that the sensilla homologs of each species respond in a similar manner. The intensity of responses to various repellents differed, indicating interspecific sensitivity differences.

Based on electrophysiologic responses, three modes of repellent action have been postulated. (1) High concentrations of repellents or other compounds, in acting on sensilla (which may show varying sensitivity to those compounds), may "jam" the central nervous system, thereby inhibiting host behavior and/or eliciting avoidance behavior. (2) Repellents eliciting responses from sensilla that are functional for behavior other than host-seeking may direct the selection of that behavior, thus inhibiting host-seeking. Behavioral tests will be necessary to support these two postulates. (3) Repellents may be competitively inhibiting the action of lactic acid (LA) on the LA-excited A3 sensilla. Deet and 612 have been shown to act in this manner. Rendering the mosquito anosmic to the LA from the host may be one of the mechanisms of repellency.

Changes in the physiologic state of _A. aegypti_ after the blood meal seemed to enhance the sensitivity of the short pointed sensilla to the oviposition attractant methyl butyrate but not to 4-methyl cyclohexanol. Qualitatively, there seemed to be a decreased sensitivity to LA 24 hours after a blood meal, but the quantitative results are not conclusive and as yet cannot support the initial finding. Our efforts in this area are being continued.

SUMMARY

Nectar- and blood-feeding and oviposition behaviors, which are important to the survival of the mosquito, depend heavily on the detection of airborne chemical signals. The objective of this research is to gain a better understanding of the basic mechanisms of chemoreception and the action of repellents on the sensory system, thus facilitating the development of more efficient means for interfering with the detection of the host or otherwise altering the behavior of the vector. The overall aim is to protect military personnel against vector-borne diseases.

The comparison of the responses of the five different types of chemosensory sensilla on the antennae of *Aedes aegypti* indicated that these different morphologic types each responded to a series of host odors, oviposition attractants, and repellents with characteristic patterns of electrophysiologic responses. Each receptor type responded to at least a few repellents, hence weakening the case for a specific nociceptor sensilla which would be sensitive to noxious stimuli and repellents. Interspecific comparisons of electrophysiologic responses of *A. triseriatus*, *Culex tarsalis*, and *Anopheles stephensi* with those of *A. aegypti* revealed that the sensilla homologs of each species respond in a similar manner. The intensity of responses to various repellents differed, indicating interspecific sensitivity differences.

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CONTENTS

INTRODUCTION

The terrestrial environment consists of a great number of chemicals, many of which impinge on the peripheral chemoreceptors of a mosquito during its life. Although many of the chemicals have little or no significance, the ability to detect those associated with nectar sources, blood-meal hosts, and oviposition sites is of vital importance to the mosquito's survival. Equally important is the detection of chemicals detrimental to those basic life processes. The objectives of our ongoing research are to develop a profile of the electrophysiologic responses to an array of chemicals for each class of chemoreceptor; to determine the interactive effects of repellents on the responses of these receptors to other chemical stimuli; and, finally, to determine the effects of changes in physiologic states on the receptors' response characteristics. Understanding the underlying mechanisms of chemoreception will ultimately make it possible to modify the mosquito's behavior.

The neurophysiologic study of the chemosensory systems of the mosquito over the past year has focused primarily on the responses of the antennal chemoreceptors to a variety of airborne chemical compounds, mainly host odors and oviposition-site attractants and repellents. A major portion of the effort was expended on *Aedes aegypti*-Masaka, and comparisons with other mosquito species (*A. triseriatus*, *Culex tarsalis*, and *Anopheles stephensi*) were made. Lactic acid (LA)-repellent interactions with the grooved-peg sensilla (A-3) were monitored quantitatively to gain some insight into the mode of action of repellents. The responses of pre-blood meal and post-blood meal *A. aegypti* females and conspecific females ready to oviposit were monitored both qualitatively and quantitatively to determine the effects of different physiologic states on the peripheral antennal chemoreceptors.

RESULTS AND DISCUSSION

Response Patterns of the Sensilla of A. aegypti-Masaka

The peripheral receptor's ability to detect a broad spectrum of chemical signals underscores the importance of the chemosensory modality to the mosquito. When presented with a wide array of chemical stimuli, the four sensilla trichodea types and the groove peg (A-3) sensilla described by McIver (1974, 1978) showed characteristically different response patterns to chemical stimuli classified according to the behavior with which they are associated (Table 1). Although the qualitative syringe stimulus delivery system used in comparing responses among sensilla types and mosquito species does not deliver equivalent doses of material, the receptors respond in a relatively consistent manner, giving strong responses (++ or --) to the compounds for which they have a high specificity and lesser (+ or -), mixed (+/-), or no (0) responses to compounds for which their specificity is weaker or lacking. The percentage increase or decrease in spike frequency from spontaneous activity levels determines the magnitude of response. A strong excitatory response is defined as a change in spike frequency (ΔF) of 300% or greater than the spontaneous activity level; a strong inhibitory response is a ΔF of 50-100% below spontaneous activity. Hence, it is possible to make a rapid semiquantitative assessment of the responses to different compounds, thus enabling the development of specificity profiles for each sensilla type and providing a basis for selecting the compounds that merit further study via the quantitative sparging system (Davis and Rebert, 1972).

Al Sensilla

With the exception of ammonia and isobutyric acid, the long pointed sensilla (Al) of A. aegypti exhibited no response to the host-related compounds that we tested. In the case of ammonia, since concentrations were considerably greater than physiologic levels, the responses may well

Table 1

SUMMARY OF THE RESPONSIVENESS
OF THE DIFFERENT TYPES OF SENSILLA
TO BEHAVIORALLY RELATED CATEGORIES OF CHEMICAL STIMULI

Stimulus Class	Sensilla Trichodea: A1	Sensilla Trichodea: A2-I Long Blunt	Sensilla Trichodea: A2-II Short Blunt	Sensilla Trichodea: Short Pointed	Grooved Peg Sensilla: LA-Excited	Grooved Peg Sensilla: LA-Inhibited
Host-related	0	0	+	0/-	++	--
Oviposition	0	+/0	++/0	++	0	0/-
Repellent	0	+/0	++	+/-	+/0	+/--
Other (nectar, misc.)	+/-	+/0	++	+/0	0	0

The table is a composite of the predominant responses elicited from A. aegypti, A. triseriatus, C. tarsalis, and An. stephensi.

Key: +, ++, increased spike frequency; -, --, decrease in spike frequency; 0, no change in spike frequency.

have been to the noxious nature of high ammonia concentrations. The A1 sensilla responded weakly or not at all to oviposition site attractants and the repellent series. Citronellol and geraniol induced a decrease in A1 spike activity, as Lacher (1967) also showed, but 612 and SRI-C6 (n-hexyl-tri-ethyleneglycol-mono-ether) had the opposite effect in that they elicited an increase in A1 spike activity.

A2 Sensilla

The other categories of sensilla trichodea, which at one time were all considered as a single type A2, have been separated into three groups (short pointed; long blunt, A2-I; and short blunt, A2-II) by McIver (1978) as the result of her morphological and ultrastructural study. Our electrophysiological results indicate that the three morphological types have different physiological characteristics. Any confusion or conflict that exists regarding responses from the A2 sensilla is probably attributable to the prior lack of morphological distinction among the sensilla types within the former broad A2 sensilla category.

The short pointed sensilla (Table 2) showed increases in spike frequency in response to all the oviposition site attractants and to a few repellents, but seemed to be inhibited (strongly in a few cases--e.g., butyric acid) by the host-related compounds. The long blunt (A2-I) sensilla, although generally being much less responsive, did respond somewhat like the short pointed sensilla in that responses to repellents and oviposition compounds were generally excitatory (+ or ++), whereas responses to host odors were absent or weakly inhibitory. In contract, the short blunt (A2-II) sensilla responded positively to most of the host odors, repellents, and oviposition attractants. Strong responses to the cresol compounds and consistently strong response to SRI-C6 distinguished this sensilla from all the others (Table 2).

A3 Sensilla

The last group, the grooved-peg (A-3) sensilla, were the most difficult to record from and generally were either excited or inhibited by lactic acid (L/). The co parative data on receptor specificity are not

Table 2

QUALITATIVE RESPONSES OF A2-TYPE SENSILLA TRICHODEA OF FOUR SPECIES OF MOSQUITOES TO CHEMICAL STIMULI

	Short Pointed				Short Blunt (A2-II)				Long Blunt (A2-I)			
Species:	*Aedes aegypti*	*Aedes triseriatus*	*Culex tarsalis*	*Anopheles stephensi*	*Aedes aegypti*	*Aedes triseriatus*	*Culex tarsalis*	*Anopheles stephensi*	*Aedes aegypti*	*Aedes triseriatus*	*Culex tarsalis*	*Anopheles stephensi*
Oviposition-related												
Ethyl lactate	++	++	++	++	-/+	0/++	0/--	++	++	+	++	++
2-butoxyethanol	++	++	++	++	++	-/+	++	++	++	++	++	++
p-Cresol	0/++	0/++	0/++	++	++	++	+/++	++	0	0/+	0	0
o-Cresol	0/+	+	0/+	++	++	++	++	++	0	0/-	0	0
m-Cresol	0/+	++/0	0/++	++	0/+	+	++/0	+	0	0	0	0/-
Ethyl propionate	0/++	0/+	0	0/+	0/+	0	0/+	0	0/++	+	--	0
Methyl propionate	0/+	0/+	0/+	0/+	+	0	0/++	0	0/+	0/+	0	0
Methyl butyrate	++	++	0	0/+	0	--	+	0	0/+	0/++	0	0/-
Ethyl acetate	+	0/++	++	--	0	--	0	--	--	0/+	++	--
4-Methylcyclohexanol	++	++	++	--	0/-	0/-/+	+/-	--	0	0/+	++	--
Repellents												
Deet	0/-	--/0	0	0/-	+	-/+	0/++	0	0/+	0/-	0	+/-
Indalone	0	++	0/++	0	+	0/++	0/++	0	0	0	0	0
612	++	-/+	0	0	++	++/0	++/+	+	0/+	0/+	0/+	0
SRI-C6	++/0	0/--	0/+	0	++	++	++	++	0	0/++	0/+	0/+
2504-5	0	--	0	--	++	0	0/++	0	0/+	+	0	0/-
Citronellol	+/0	0/--	0/+	+/-	0/++	++	++	++	0	+	0/+	++
Geraniol	-/0	0/--	0/+	--/++	++	0	0/++	0	0	+/0	0	+
Naphthalene	0	0	0/+	+	+/0	0	+	++	0	0	0	0
Juglone	+	0	0	--	0	0	--	--	0/+	+	--	--

Key: +,++, increased spike frequency; -,--,decrease in spike frequency; 0, no change in spike frequency.

as complete as they could be because quantitative (sensitivity) dose-response relationships and repellent interactions--rather than qualitative data--were usually sought when a good electrode placement was achieved. However, the data (Table 3) do seem to indicate that the LA-inhibited sensilla were generally inhibited by the LA analogs as well as by most oviposition-related stimuli and a few of the repellents. The converse was true for the LA-excited sensilla with the exception of oviposition-related compounds, which tended to be totally unresponsive or inhibited, as in the case of ethyl lactate. In general, only a small percentage of A-3 sensilla responded strongly to either repellents (in 13% of trials) or oviposition-related substances (in 11% of trials).

Thus, the broad spectrum qualitative screen using a set series of chemical compounds has revealed a definite pattern of responses for each sensilla type. Testing each sensilla type with the entire series of compounds is an important facet of our mosquito chemoreception paradigm since any one of these chemicals will impinge on all the sensilla when the mosquito enters its presence. It is important to note that the host compounds tend to inhibit the responses of the short pointed sensilla, which respond strongly to the oviposition attractants. This indicates that the peripheral receptors play a major role in the determination of which behaviors may result when a mosquito encounters certain stimuli. When a female is actively seeking a blood meal, the sensory information dealing with oviposition site location is suppressed. Thus, the same stimulus acting in different ways on different sensilla may promote one behavior over another. In the section on the effects of physiologic states, we will discuss how these sensitivities may, in part, be reversed following the successful attainment of a blood meal.

Interspecific Comparison

The corresponding sensilla types on the antennae of A. triseriatus, C. tarsalis, and An. stephensi exhibited physiologic response characteristics similar to those of their sensilla homologs on the antennae of A. aegypti (Tables 1, 2, and 3). The A1 sensilla of the four species were all relatively unresponsive to the array of chemicals. The short pointed

Table 3

QUALITATIVE RESPONSES OF GROOVED-PEG SENSILLA
OF FOUR SPECIES OF MOSQUITOES TO CHEMICAL STIMULI

Sensillar Responses

Stimuli / Species:	LA-Inhibited Sensilla				LA-Excited Sensilla			
	Aedes aegypti	*Aedes triseriatus*	*Culex tarsalis*	*Anopheles stephensi*	*Aedes aegypti*	*Aedes triseriatus*	*Culex tarsalis*	*Anopheles stephensi*
Host-Related								
Lactic acid	--	--	-	--	++	++	+	++
Glyceric acid	--	0/--			0/++			
2-Br-Propionic acid	0/--	-	0	0		+		
2-Cl-Propionic acid	--/++	--	-	--	++	++		+
Butyric acid	++	--/-	0	--	--	++/-		0
Isobutyric acid	++	--	+	--	++/-	++/--		--
2-OH-isobutyric acid	-	-		0	++	0		
2200-10	0	-		0	0			+
Acetic acid	-	--		0	0			
Water vapor		-		0	0	0		
Ammonia	++	++			++	++/--	+	
Repellents								
Deet	0/++	0/-		-	0	++/0	++	0
Indalone	+	-		-	+	0		0
612	-	0/++		--	++/0	+	+	0
SRI-C6	-	0/--		-	-/++	++	0	0
2504-5	0/-	0		0	0	0		
Citronellol	0/++	0		-	0			
Geraniol	0	-		-	0	0		
Naphthalene	0	0		0	0			0
Juglone	+	+		++	++			

Key: +, ++, increased spike frequency; -, --, decrease in spike frequency; 0, no change in spike frequency.

sensilla generally showed strong excitatory responses to oviposition attractants, variable responses to repellents, and inhibitory responses to some of the host compounds. Although much less responsive overall, the long blunt A2-I sensilla all showed a response pattern similar to that of the short pointed sensilla. Nearly all the short blunt (A2-II) sensilla exhibited an excitatory response to the array of compounds and all showed the characteristically strong response to SRI-C6 and to the cresols. The assay of the grooved-peg (A-3) sensilla is not yet complete; however, based on comparisons of the response of A. aegypti with the existing data on the other mosquitoes, we do not anticipate any major differences in the patterns of response. Sensilla homologs from the four species of mosquitoes examined have similar qualitative electrophysiologic response properties.

Although interspecific similarities were readily apparent [e.g., in the strong responses to ethyl lactate and 2-butoxyethanol by the short pointed sensilla of all four species of mosquitoes (Table 2)], differences in response intensity and consistency of responses were also evident. The short pointed sensilla of the four species all responded to o-cresol with increasing spike frequency, but the responses of A. aegypti and C. tarsalis were weak and inconsistent (0/+) whereas those of A. triseriatus and An. stephensi were, respectively, consistently weak (+) and consistently strong (++). Qualitative interspecific differences were most evident in the mosquitoes' response to repellents. Deet elicited either excitatory, inhibitory, mixed, or no responses--depending on sensilla type and species. Therefore, the responses of the sensilla homologs to oviposition and host compounds, although varying in intensity and consistency, were generally of the same quality, whereas the responses to repellents had interspecific fluctuations in quality and varied in intensity and consistency.

An. stephensi does present somewhat of a problem because, aside from the fact that the great mass of sensilla makes it more difficult to distinguish among them, Boo (in press) had indicated that there are five types of sensilla trichodea. Boo's types A, E, and D seem to correspond both morphologically and physiologically to the long pointed, short pointed,

and short blunt of the other species, but questions remain in regard to type C (which seems to correspond to the long blunts) and the additional type B.

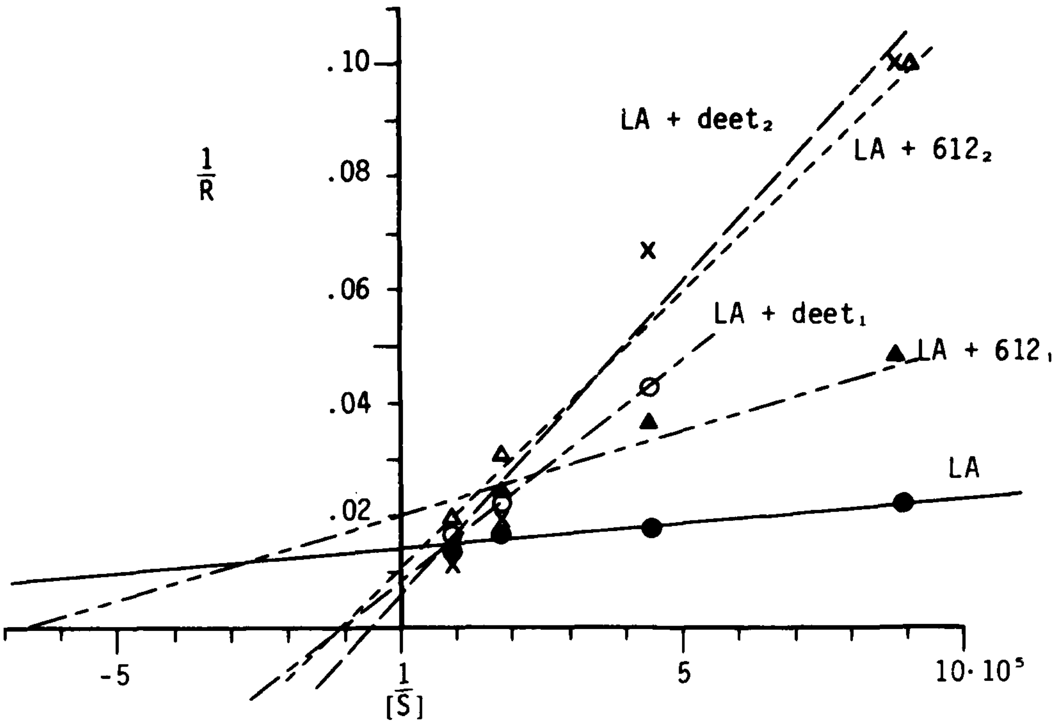
Actions of Repellents

In spite of vector control measures, the ubiquitous mosquito persists, necessitating the use of repellents as a measure of protection. A repellent, if effective, prevents the mosquito from taking a blood meal, but the mechanism of this repellency is little understood. Most of the sensilla on the antennae of the four species of mosquitoes responded to at least a few repellents. However, in many cases the responses were relatively weak or inconsistent. Strong responses to specific repellents--especially SRI-C6, 612, and citronellol--were most consistently recorded from the A2-II sensilla. However, many of the other sensilla types responded to one or more of the repellents with sufficient intensity to question the notion of a noxious substance--"repellent"--receptor-role previously assigned to certain A2 sensilla types (Davis and Rebert, 1972). In addition, the sensilla that exhibited the strongest response to the repellents--the short blunt A2-II--also responds strongly to oviposition site attractants, host-related odors, and some other plant-related stimuli. These qualitative results suggest alternative modes of action for some of these repellents. For example, it is possible that the responses of two or more types of sensilla to a repellent results in the "jamming" of the central nervous system (CNS) with inappropriate information, thereby inhibiting host-seeking and/or eliciting avoidance behavior. This hypothesis is supported by information that p-cresol functions as an oviposition attractant at low concentrations and as a repellent at higher concentrations (Bentley, personal communication) and that LA, the host attractant, repels at higher concentrations (Wright, Kellogg, and Burton, 1969). A second possibility is that the repellents, which elicit responses from the oviposition attractant-sensitive short pointed and A2-II sensilla, may act in the selection of oviposition behavior and thus inhibit host-seeking.

A third hypothesis may, in part, account for the effectiveness of deet as a repellent. Although deet is one of the most effective and widely used repellents, none of the sensilla showed a strong response to it, thereby suggesting another mode of action for repellents than those discussed above. Davis and Sokolove (1976) found that the response of the LA-excited A-3 sensilla to LA was attenuated by deet. To study this effect in more detail, LA was presented alone and together with different intensities of deet and 612. The response (change in spike frequency for individual LA-excited neurons) to LA and to LA plus repellent was plotted against the stimulus intensity. Both deet and 612 shifted the response curves to the right, toward the higher intensities of LA necessary to elicit a given level of response. Although the differences were not great, deet appeared to cause a slightly greater shift than did 612. This shifting of the response curves was dose-dependent and suggests that the repellents in some way inhibit the response of the sensory neurons to LA.

To assess what type of inhibitory processes might be involved and to determine some of the kinetic properties of the LA-excited neurons, we replotted the data using a modified Lineweaver-Burke double-reciprocal plot of response as a function of stimulus intensity (Figure 1). The resulting curves indicated that deet, at the intensities used (2.1 and 5.3×10^{-7} moles/min), acted as a competitive inhibitor of LA in the LA-excited neuron. At 5.3×10^{-7} moles/min, the repellent 612 also inhibited the action of LA in a competitive manner. At the lower stimulus intensity of 2.1×10^{-7} moles/min, 612 appeared to be either noncompetitive or intermediate between competitive and noncompetitive in its interaction with LA. However, more recent data suggest that 612 at the lower intensity may competitively inhibit the LA-excited response. This issue will be resolved only by replication of these studies at slightly different 612 intensities.

In addition, for the LA-excited neuron, the double-reciprocal plots revealed a range of K_D values between 3×10^{-8} and 4×10^{-6} for LA alone. K_D values in this range are typical for enzymatic processes. We are continuing these experiments using other repellents of known behavioral effectiveness.



LEGEND:

	LA alone	LA + deet$_1$ at $2.1 \cdot 10^{-7}$ moles/min	LA + deet$_2$ at $5.3 \cdot 10^{-7}$ moles/min	LA + 612$_1$ at $2.1 \cdot 10^{-7}$ moles/min	LA + 612$_1$ at $5.3 \cdot 10^{-7}$ moles/min
Stimulus:	●——●	o- - - -o	x— — —x	▲- - - -▲	△- - - -△
K_D :	$7.1 \cdot 10^{-7}$	$9.1 \cdot 10^{-6}$	$1.7 \cdot 10^{-5}$	$1.5 \cdot 10^{-6}$	$8.3 \cdot 10^{-6}$
Rmax :	71	111	143	50	83
Correlation coefficient:	0.938	0.998	0.979	0.981	0.999
Type of Inhibition :	--	Competitive	Competitive	Intermediate: Noncompetitive-Competitive	Competitive

R = Response defined as the change in spike frequency in impulses/sec.

[S] = Stimulus intensity in moles/min.

All curves fitted to data using linear regression technique.

FIGURE 1 DOUBLE-RECIPROCAL PLOT OF THE RESPONSE OF A LA-EXCITED NEURON TO LA ALONE AND TOGETHER WITH DEET OR 612

It should be noted that the term "repellent," when used to refer to a substance that prevents a mosquito from biting a host organism, is an operational definition and does not refer to a specific behavior pattern or taxis (Dethier, 1960) that the "repellent" substance may elicit. Because of this operational use of the term "repellent," the mode(s) of action of these compounds are not understood and may, in fact, vary between different compounds. In the search for better "repellents," all possibilities--from a noxious substance receptor to elicitation of a competing behavior pattern or the production of an anosmic state--must be considered.

Effect of Changes in the Physiologic State on Receptor Response of A. aegypti

Since host-seeking is inhibited during oocyte maturation (Klowden and Lea, 1979), we monitored the effects of this blood meal-induced change in physiologic state on the response of the antennal chemoreceptors to our series of host- and oviposition site-related compounds and repellents. To study this effect, male and female A. aegypti mosquitoes were transferred at weekly intervals from the rearing tank cages to smaller blood-feeding cages and maintained for one week on 5% sucrose solution. After being starved for 24 hours, the females, which were presumed to have been inseminated by then, were allowed to feed to repletion on the shaved abdomen of a guinea pig. Electrophysiologic responses to chemical stimuli were recorded at 24-hour intervals for three days after the blood meal; the stimuli were either presented neat through a 10-cc syringe in an attempt to monitor qualitative changes or presented in quantitative doses in an increasing series, using the air sparging system in order to study dose-response relationships.

In our Quarterly Progress Report No. 5 (15 May 1979) we concluded that the qualitative electrophysiologic data did not reveal any differences between pre- and post-blood meal states that could account for the reported inhibition of host-seeking behavior. However, additional data obtained since then coupled with a more critical analysis of the combined data indicated that perhaps the opposite was true. The LA-excited A3 sensilla of control females responded strongly (++) to LA, but responded weakly

(+) to LA at 24 hours, recovering to a strong LA response (++) at 48 and 72 hours (see Section D in Table 4). With the range of responses displayed in a histogram, it was evident that the strong responses decreased about 46% from control values (see A in Figure 2).

The data from the short pointed sensilla (C in Table 4) show that post-blood meal females are more sensitive to the fatty acid esters (methyl propionate, methyl butyrate, and ethyl acetate) than the controls and that there was little or no change in sensitivity to other oviposition-related compounds such as ethyl lactate and 4-methyl cyclohexanol. Methyl butyrate (B in Figure 2) and ethyl acetate elicited the greatest percentage of strong responses at 48 hours post-blood meal, a time at which A. aegypti normally oviposits. Methyl propionate did not elicit any strong responses until 48 hours post-blood meal, but the responses remained at the same high level at 72 hours. This is a significant finding, considering that the fatty acid esters used have been reported to be oviposition attractants for A. aegypti females (Perry and Fay, 1967). Control females allowed access to oviposition sites deposited their eggs between 48 and 72 hours. Egg-laying was triggered in some of the blood-fed mosquitoes during the electrophysiologic recording sessions. The sensitivity of the short pointed sensilla to ethyl lactate remained unchanged in samples from pre- and post-blood meal females, but the strong responses to 4-methyl cyclohexanol declined at 48 and 72 hours (C in Figure 2). This is additional support for a change in sensitivity for those stimuli specific to A. aegypti; e.g., ethyl lactate is only a very weak attractant to A. triseriatus, with no effect on A. aegypti, and 4-methyl cyclohexanol is an analog of p-cresol, an oviposition attractant in A. triseriatus (Bentley et al., in press).

Although we do not yet have enough data to permit statistical verification of the observed differences, the qualitative data establish a definite trend showing that the sensitivities of the neurons of the short pointed and--most likely--the grooved-peg sensilla to specific behaviorally active compounds were altered following a blood meal by the female. Our data now available are stronger for the oviposition site-sensitive neurons of the short-pointed sensilla than for the LA-sensitive neurons.

Table 4

QUALITATIVE RESPONSES OF THE ANTENNAL SENSILLA
OF *A. aegypti*-Masaka
TO SELECTED STIMULI FOLLOWING A BLOOD MEAL

			Sensillar Responses				
				Post-Blood Meal			
	Sensilla	Stimulus	Pre-Blood Meal (Control)	24 hr	43 hr	72 hr	Net Change in Sensitivity
A.	A2-I S. trichodea	Butyric Acid	0	0	--	0	Increase
		Deet	0/+	0	+	0	No change
		Ethyl lactate	+	++	++	+	Increase
B.	A2-II Short blunt S. trichodea	Deet	+/0	+/0	+/0	+/0	No change
		Naphthalene	0	++	++		Increase
		p-cresol	0/++	+	++		Increase
		Ethyl propionate and other fatty acid esters	0/+	0/+/-	-/0/+		No change
C.	Short pointed S. trichodea	Deet	0/-	0	+	0	No change
		Indalone	0	+	+	++/0	Increase
		612	++/0	++	0	+	Decrease
		SRI-C6	++/0	+	0	0	Decrease
		Citronellol	+/0	++	+	+	Increase
		Ethyl lactate	++	++	++	++	No change
		Cresols	0/+	0	0	0	No change
		Fatty acid esters	+/++	0	++	++	Increase
D.	Grooved-peg: LA-inhibited	Lactic acid	--	--	-	--	No change
		Butyric acid	++	++/--	--/++	--	Qualitative change
	LA-excited	Lactic acid	++	+	++	++	No change
		Butyric acid	--	--/++		++/-	Qualitative change

Key: +, ++, increased spike frequency; -,--, decrease in spike frequency;
0, no change in spike frequency.

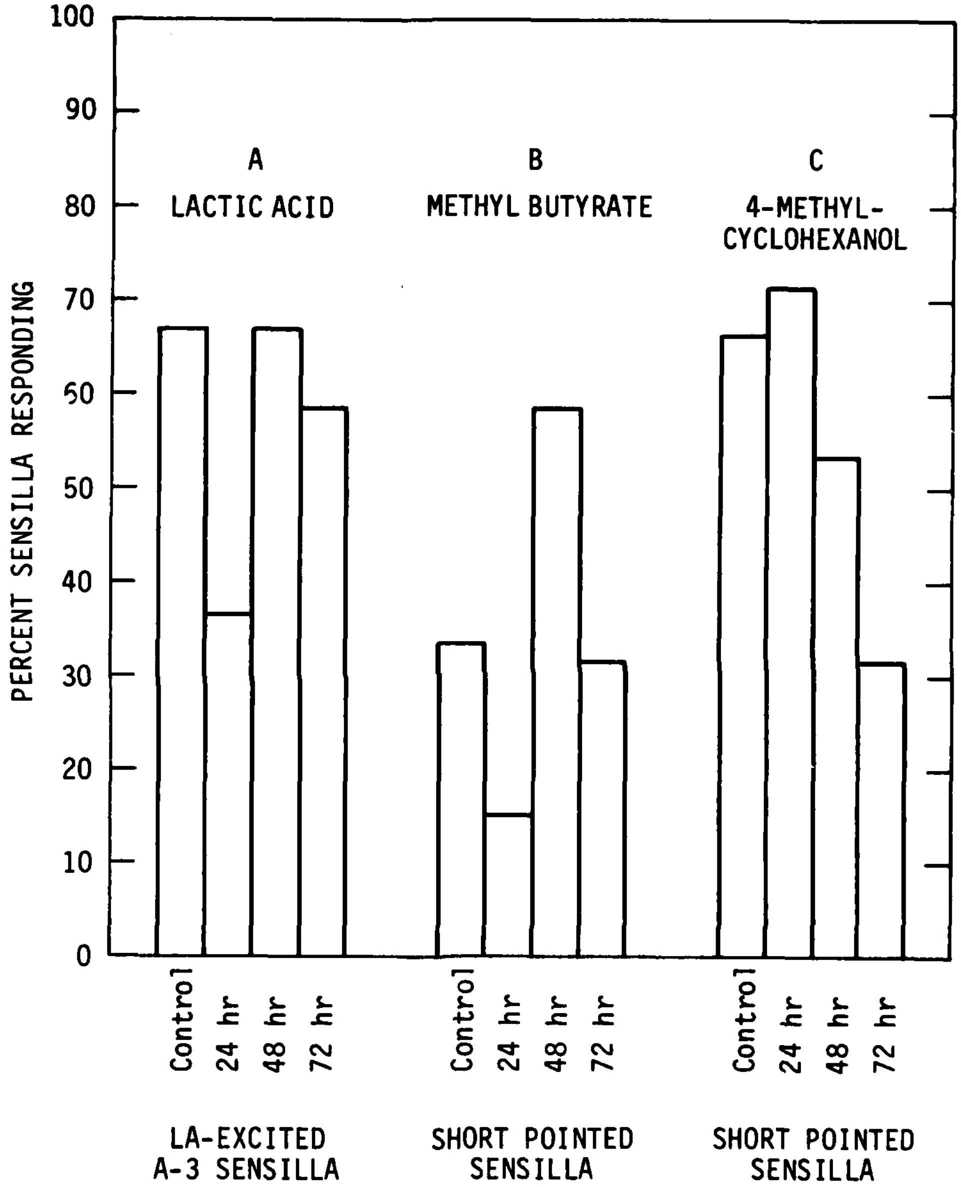


FIGURE 2 PERCENTAGE OF SENSILLA FROM PRE- AND POST-BLOOD MEAL FEMALE MOSQUITOES SHOWING STRONG (++) RESPONSES TO LA, METHYL BUTYRATE, AND 4-METHYLCYCLOHEXANOL.

To obtain quantitative information on changes in receptor sensitivity at stimulus intensities nearer physiologic levels, we monitored the effects of LA, 4-methyl cyclohexanol, and methyl butyrate (each in a graded intensity series) on the antennal chemoreceptors sensitive to these substances. Lactic acid is a well-documented host odor (Wright et al., 1969) and methyl butyrate has been reported to be a strong oviposition attractant (Perry and Fay, 1967). 4-Methyl cyclohexanol, a structural analog of p-cresol, can act as an oviposition site attractant for A. triseriatus but is not active with A. aegypti, according to Bentley (personal communication). We used 4-methyl cyclohexanol because it elicits a very robust response from the short pointed sensilla. Bentley has not identified this compound in tree-hole water, the natural oviposition site of A. triseriatus.

The quantitative response data ("dose response" curves) for the LA-excited neurons show a normal range of variability and the curves for pre- and post-blood meal overlap. However, even though the individual dose-response curves of pre- and post-blood meal mosquitoes overlap, the mean responses of the pre-blood-fed mosquitoes at the higher stimulus intensity were greater than the corresponding mean responses of the post-blood meal mosquitoes (Figure 3). This preliminary experiment suggests that there may be a decreased sensitivity in the receptor response following a blood meal.

The normal variation in receptor sensitivity precludes our ability to make a definitive statement regarding changes in receptor response characteristics. This can only be resolved by sampling a larger population of receptors or by modifying our procedure to reduce this apparent variability--e.g., we could determine presence of sperm and the stage of ovarian development so that physiologic states can be more accurately assessed. This may be necessary since 35% of the females given blood enemas failed to develop mature eggs and continued host-seeking and since gravid but uninseminated females showed less host-seeking inhibition than did gravid and inseminated females (Klowden and Lea, 1979). To complicate matters, there are two types of LA-sensitive neurons; hence, there is only a 50% probability that the electrode would be positioned next to a LA-excited rather than a LA-inhibited receptor cell.

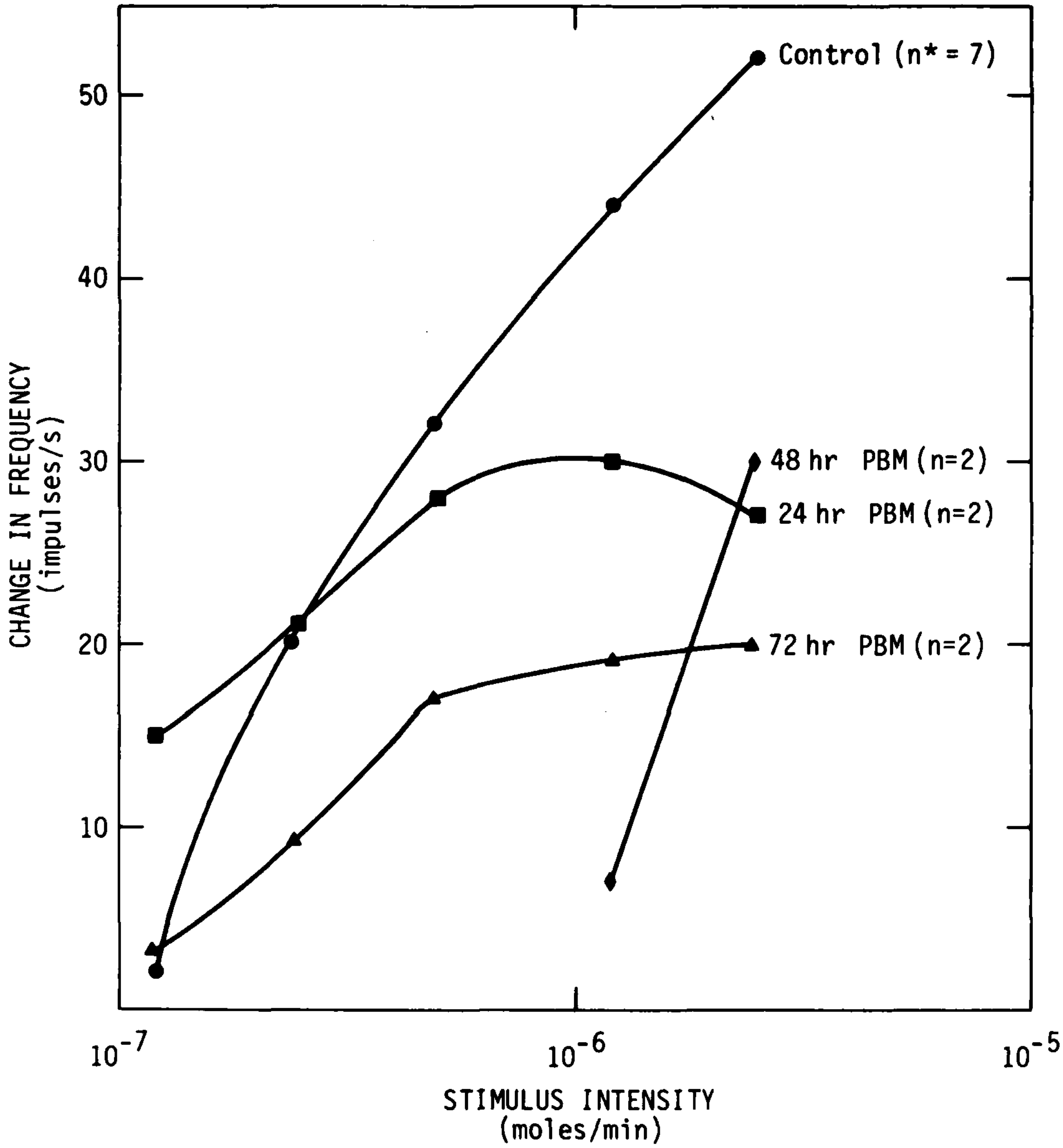


FIGURE 3 RESPONSE OF THE LA-EXCITED NEURONS TO LA IN <u>A. aegypti</u> FEMALES PRE-BLOOD MEAL (CONTROL) AND 24, 48, AND 72 HOURS POST-BLOOD MEAL (PBM). n* INDICATES NUMBER OF REPLICATES AVERAGED.

The responses of the short pointed sensilla to methyl butyrate (Figure 4) show that the sensitivity is most enhanced at 48 hours post-blood meal and that sensitivities at 24 and 72 hours post-blood meal range between the control and the 48-hour high value. These results correlate well with the qualitative results (B in Figure 2), which indicate the greatest sensitivity at 48 hours and little change in sensitivity at 24 and 72 hours. The one 24-hour post-blood meal dose-response that was rather high may mean that the physiologic conditions of that mosquito were approaching that of the 48-hour post-blood meal state due to normal variability in the rates of development and time of recording.

As with methyl butyrate, there was a close correlation between the quantitative and qualitative data obtained in the study of the effects of 4-methyl cyclohexanol on the short pointed sensilla. The dose-response curves showing the effect of the latter compound on the short pointed sensilla of pre- and post-blood-fed mosquitoes were distributed randomly above and below those of the controls. Although the one-way analysis of variance of mean frequencies for females pre-blood meal and 24, 48, and 72 hours post-blood meal indicated no significant difference ($P > 0.05$) between the responses of the groups tested, comparison of the mean receptor responses at 48 and 72 hours post-blood meal were mildly depressed at the higher concentrations. This depressed activity explains the apparent decrease in strong responses to this compound at 48 and 72 hours post-blood meal (C in Figure 2) during the qualitative experiments. Neat concentrations of 4-methyl cyclohexanol delivered by means of the 10-cc syringe during qualitative testing were probably fairly high and therefore the responses recorded from the sensilla 48 and 72 hours post-blood meal were probably similarly depressed. The significance of this inhibition is not apparent since as far as *A. aegypti* is concerned, no functional role has yet been ascribed to this compound.

Correlation of Electrophysiologic Results with the Behavior of the Mosquito

Klowden and Lea (1979) first reported a hemolymph-borne factor, recently defined as ecdysone (Beach, 1979), that inhibits host-seeking behavior in blood-fed female mosquitoes. Whether a factor such as ecdysone

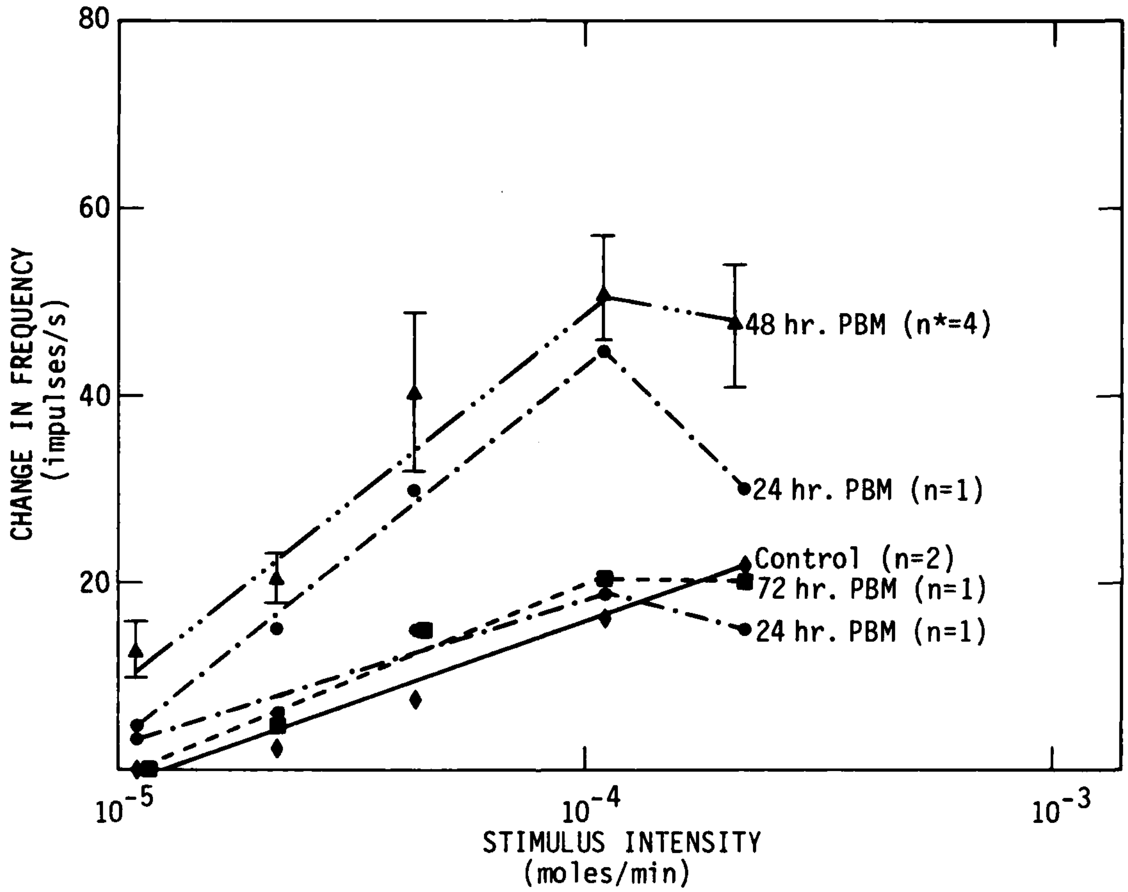


FIGURE 4 RESPONSE OF THE SHORT POINTED SENSILLA TO METHYL BUTYRATE IN A. aegypti FEMALES PRE-BLOOD MEAL (CONTROL) AND 24, 48, AND 72 HOURS POST-BLOOD MEAL (PBM). *n INDIATES NUMBER OF REPLICATES AVERAGED

exerts its influence in the insect's CNS or at the peripheral receptor level has not been determined. Certainly, the changes we have observed in the sensitivities of certain antennal sensory neurons would suggest a peripheral site of action.

If stimulus specification occurs at the receptor level and the receptor sensitivities are enhanced for the "correct" oviposition attractant(s) (as our results with methyl butyrate suggest), and if sensitivities are not enhanced for oviposition attractants of another species, we may infer that this is a mechanism by which different species can select different oviposition sites, especially since our data show that homologous sensilla of different species of mosquitoes respond to the same compounds in our test series. The "de novo" appearance of a different receptor site that would be sensitive to attractants unique to certain oviposition sites is less probable than is the "tuning" of a receptor already on the ancestral mosquito. Enhanced sensitivity to attractants characteristic of certain oviposition sites may have been one of the factors involved in the speciation of mosquitoes.

The study of the effect of changes in the physiologic state on the receptor response is not yet complete, and most of the remaining two quarters of the contract year will be spent replicating experiments in order to confirm (or refute) our present, tentative, conclusions. Results from these diverse experiments are beginning to fit into a pattern that may eventually develop into a model for mosquito chemoreception. The changes in physiologic states do seem to cause changes in spike frequency, which reflect receptor sensitivity changes. However, changes in frequency alone may not be a sensitive measure of changes occurring in the receptor. In the future, we will broaden our response parameters to include changes in the rate of response, saturation, and thresholds. The inhibition of spike frequency in response to the higher concentrations of 4-methyl cyclohexanol and, to a lesser extent, methyl butyrate in post-blood meal mosquitoes must be looked at more closely since this effect, which is not apparent in the pre-blood meal females, could indicate changes in receptor physiology. Although no definite conclusions have been drawn, we can now expand and modify our mosquito chemoreception

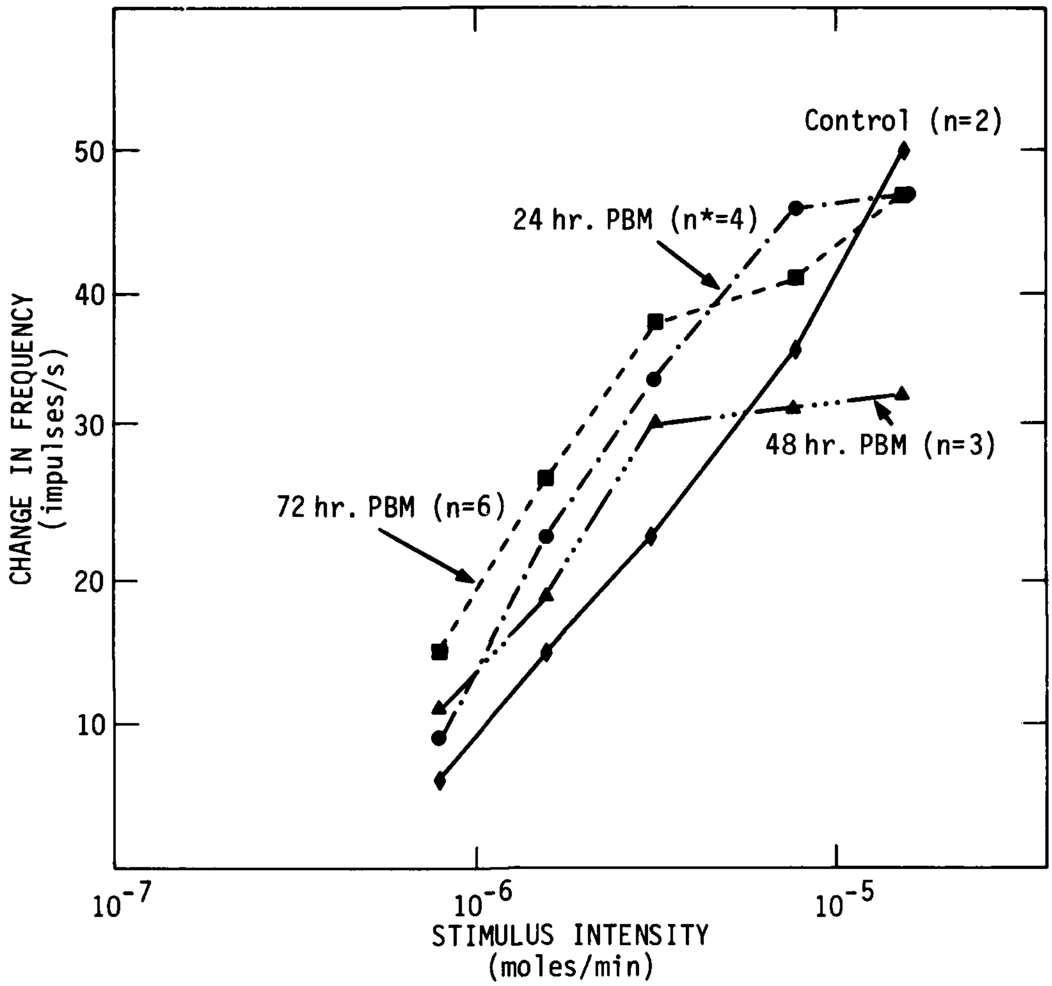


FIGURE 5. MEAN RESPONSE OF THE SHORT POINTED SENSILLA TO 4-METHYL CYCLOHEXANOL IN A. aegypti FEMALES PRE-BLOOD MEAL (CONTROL) AND 24, 48, AND 72 HOURS POST-BLOOD MEAL (PBM). *n INDICATES NUMBER OF REPLICATES AVERAGED

paradigm to include changes in receptor sensitivity. To obtain the complete picture, future study must include inputs from other chemosensory systems, such as the contact chemoreceptors.

FUTURE PLANS

In the next quarter, we will:

(1) Continue studying the effect of the blood meal-induced change in physiologic state on the chemoreceptor responses with experiments designed to resolve ambiguities.

(2) Continue to monitor, both qualitatively and quantitatively, the responses of sensilla of various species to host and oviposition site attraction compounds and repellents.

(3) Initiate experiments to record the electrophysiologic responses of contact chemoreceptors on the labial palps and tarsi of *A. aegypti* to various chemical stimuli.

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